FILE 'HOME' ENTERED AT 14:53:29 ON 28 JUN 1997

=> file biosis, medline, embase, wpids

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	0.15	0.15

FILE 'BIOSIS' ENTERED AT 14:53:50 ON 28 JUN 1997

FILE 'MEDLINE' ENTERED AT 14:53:50 ON 28 JUN 1997

FILE 'EMBASE' ENTERED AT 14:53.50 ON 28 JUN 1997

FILE 'WPIDS' ENTERED AT 14:53:50 ON 28 JUN 1997

=> s vegf or vascular endothelial (1a) growth factor
L1 1553 FILE BIOSIS
L2 811 FILE MEDLINE
L3 851 FILE EMBASE
L4 67 FILE WPIDS

TOTAL FOR ALL FILES
L5 3282 VEGF OR VASCULAR ENDOTHELIAL (1A) GROWTH FACTOR

=> s monomer
L6 11869 FILE BIOSIS
L7 9730 FILE MEDLINE
L8 8822 FILE EMBASE
L9 89179 FILE WPIDS

TOTAL FOR ALL FILES
L10 119600 MONOMER

=> s l10 and l5
L11 2 FILE BIOSIS
L12 2 FILE MEDLINE
L13 2 FILE EMBASE
L14 1 FILE WPIDS

TOTAL FOR ALL FILES
L15 7 L10 AND L5

=> duplicate remove l15
DUPLICATE PREFERENCE IS 'BIOSIS, MEDLINE, EMBASE, WPIDS'
KEEP DUPLICATES FROM MORE THAN ONE FILE? Y/(N):n
PROCESSING COMPLETED FOR L15
L16 3 DUPLICATE REMOVE L15 (4 DUPLICATES REMOVED)

=> d 1-

L16 ANSWER 1 OF 3 WPIDS COPYRIGHT 1997 DERWENT INFORMATION LTD
AN 97-099920 [09] WPIDS
DNC C97-031900
TI Activating cell surface receptors using peptide dimer agonists - also, new dimers of erythropoietin receptor binding peptide(s) useful for treating patient having disorder characterised by EPO deficiency.
DC B04
IN JOHNSON, D L; ZIVIN, R A
PA (ORTH) ORTHO PHARM CORP; (JOHJ) JOHNSON & JOHNSON
CYC 70
PI WO 9640772 A2 961219 (9709)* EN 110 pp C07K014-505
RW: AT BE CH DE DK EA ES FI FR GB GR IE IT KE LS LU MC MW NL OA
PT SD SE SZ UG
W: AL AM AT AU AZ BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE
HU IS JP KE KG KP KR KZ LK LR LS LT LU LV MD MG MK MN MW MX
NO NZ PL PT RO RU SD SE SG SI SK TJ TM TR TT UA UG US UZ VN
AU 9661007 A 961230 (9716) C07K014-505
ADT WO 9640772 A2 WO 96-US9469 960606; AU 9661007 A AU 96-61007 960606
FDT AU 9661007 A Based on WO 9640772
PRAI US 95-484135 950607
IC ICM C07K014-505
ICS A61K038-18

L16 ANSWER 2 OF 3 BIOSIS COPYRIGHT 1997 BIOSIS
DUPLICATE 1
AN 96:216277 BIOSIS
DN 98772406
TI The carboxyl-terminal domain (111-165) of ***vascular*** ***endothelial*** ***growth*** ***factor*** is critical for its mitogenic potency.
AU Keyt B A; Berleau L T; Nguyen H V; Chen H; Heinsohn H; Vandlen R; Ferrara N
CS Dep. Cardiovascular Res., Genentech, Inc., 460 Point San Bruno Blvd., South San Francisco, CA 94080, USA
SO Journal of Biological Chemistry 271 (13). 1996. 7788-7795. ISSN: 0021-9258
LA English

L16 ANSWER 3 OF 3 BIOSIS COPYRIGHT 1997 BIOSIS
DUPLICATE 2
AN 96:151487 BIOSIS
DN 98723622
TI Identification of ***vascular*** ***endothelial*** ***growth*** ***factor*** determinants for binding KDR and FLT-1 receptors: Generation of receptor-selective ***VEGF*** variants by site-directed mutagenesis.
AU Keyt B A; Nguyen H V; Berleau L T; Duarte C M; Park J; Chen H; Ferrara N
CS Dep. Cardiovascular Res., Genentech Inc., 460 Pt. San Bruno Blvd., South San Francisco, CA 94080, USA
SO Journal of Biological Chemistry 271 (10). 1996. 5638-5646. ISSN: 0021-9258
LA English

=> d 1- abs

L16 ANSWER 1 OF 3 WPIDS COPYRIGHT 1997 DERWENT INFORMATION LTD
AN 97-099920 [09] WPIDS
AB WO 9640772 A UPAB: 970228
The following methods are claimed: (1) (a) improving the bioactivity of a cell surface receptor, or (b) activating a cell surface receptor to induce its biological activity, comprising dimerising a monomeric agonist of the cell surface receptor, and contacting the formed dimer with the cell surface receptor; and (2) preparing a cell surface receptor agonist, comprising dimerising a cell surface antagonist. Also claimed are peptide dimers comprising 2 monomers of 10-40 aminoacids that bind to erythropoietin (EPO) receptor, with each ***monomer*** comprising a sequence of amino acids of formula (I): Xaa3-Xaa4-Xaa5-Gly-Pro-Xaa6-Thr-Trp-Xaa7-Xaa8 (I), Xaa3 = Cys, Xaa4 = Arg, His, Leu or Trp, Xaa5 = Met, Phe or Ile, Xaa6 = any natural L-amino acid, Xaa7 = Asp, Glu, Ile, Leu or Val, and Xaa8 = Cys.
USE - The new methods are useful for treating disorders resulting from deficiencies of biological factors such as EPO, GH, PDGF, EGF, GCSF, TPO, ***VEGF*** , FGF, insulin, IL3, IL5, IL6 and IL2 by improving activity of the appropriate cell surface receptor. Specifically, the claimed peptide dimers can be used to treat a patient having a disorder characterised by an EPO deficiency, or a low or defective red blood cell population (claimed). They can also be used for promoting wound healing, treating trauma or growth of collateral coronary blood vessels (such as those that occur after myocardial infarction) and for post vascular graft treatment. The dimers may also be useful for treating neurological disorders, generally characterised by low absolute levels of acetylcholine or low relative levels of acetylcholine as compared to other neuroactive substances, e.g. neurotransmitters.
ADVANTAGE - The dimer peptide exhibits increased biological potency in vitro and in vivo relative to the monomeric agonists from which dimers are derived. Moreover, cell surface receptor antagonists can be converted to cell surface receptor agonists.
Dwg.0/9

L16 ANSWER 2 OF 3 BIOSIS COPYRIGHT 1997 BIOSIS
DUPLICATE 1
AN 96:216277 BIOSIS
AB ***Vascular*** ***endothelial*** ***growth***

factor (***VEGF***) is a potent and specific mitogen for endothelial cells. ***VEGF*** is synthesized and secreted by many differentiated cells in response to a variety of stimuli including hypoxia. ***VEGF*** is expressed in a variety of tissues as multiple homodimeric forms (121, 165, 189, and 206 amino acids/ ***monomer***) resulting from alternative RNA splicing. ***VEGF*** -121 is a soluble mitogen that does not bind heparin; the longer forms of ***VEGF*** bind heparin with progressively higher affinity. The higher molecular weight forms of ***VEGF*** can be cleaved by plasmin to release a diffusible form(s) of ***VEGF*** . We characterized the proteolysis of ***VEGF*** by plasmin and other proteases. Thrombin, elastase, and collagenase did not cleave ***VEGF*** , whereas trypsin generated a series of smaller fragments. The isolated plasmin fragments of ***VEGF*** were compared with respect to heparin binding, interaction with soluble ***VEGF*** receptors, and ability to promote endothelial cell mitogenesis. Plasmin yields two fragments of ***VEGF*** as indicated by reverse phase high performance liquid chromatography and SDS-polyacrylamide gel electrophoresis: an amino-terminal homodimeric protein containing receptor binding determinants and a carboxyl-terminal polypeptide which bound heparin. Amino-terminal sequencing of the carboxyl-terminal peptide identified the plasmin cleavage site as Arg-110-Ala-111. A heterodimeric form of ***VEGF*** -165/110, was isolated from partial plasmin digests of ***VEGF*** -165. The carboxyl-terminal polypeptide (111-165) displayed no affinity for soluble kinase domain region (KDR) or Fms-like tyrosine kinase (FLT-1) receptors. The various isoforms of ***VEGF*** (165,165/110, 110, and 121) bound soluble kinase domain region receptor with similar affinity (approximately 30 pM). In contrast, soluble FLT-1 receptor differentiated ***VEGF*** isoforms (165, 165/110, 110, and 121) with apparent affinities of 10, 30, 120, and 200 pm, respectively. Endothelial cell mitogenic potencies of ***VEGF*** -110 and ***VEGF*** -121 were decreased more than 100-fold compared to that of ***VEGF*** -165. The present findings indicate that removal of the carboxyl-terminal domain, whether it is due to alternative splicing of mRNA or to proteolysis, is associated with a significant loss in bioactivity.

L16 ANSWER 3 OF 3 BIOSIS COPYRIGHT 1997 BIOSIS
DUPLICATE 2
AN 96:151487 BIOSIS
AB ***Vascular*** ***endothelial*** ***growth*** ***factor*** (***VEGF***) expression in various cell types is induced by hypoxia and other stimuli. ***VEGF*** mediates endothelial cell proliferation, angiogenesis, vascular growth, and vascular permeability via the endothelial cell receptors, kinase insert domain-containing receptor (KDR)/fetal liver kinase 1 (Flk-1) and FLT-1. Alanine-scanning mutagenesis was used to identify a positively charged surface in ***VEGF*** that mediates binding to KDR/Flk-1. Arg-82, Lys-84 and His-86, located in a hairpin loop, were found to be critical for binding KDR/Flk-1, while negatively charged residues, Asp-63, Glu-64, and Glu-67, were associated with FLT-1 binding. A ***VEGF*** model based on PDGFb indicated these positively and negatively charged regions are distal in the ***monomer*** but are spatially close in the dimer. Mutations within the KDR site had minimal effect on FLT-1 binding, and mutants deficient in FLT-1 binding did not affect KDR binding. Endothelial cell mitogenesis was abolished in mutants lacking KDR affinity; however, FLT-1 deficient mutants induced normal proliferation. These results suggest dual sets of determinants in the ***VEGF*** dimer that cross-link cell surface receptors, triggering endothelial cell growth and angiogenesis. Furthermore, this mutational analysis implicates KDR, but not FLT-1, in ***VEGF*** induction of endothelial cell proliferation.

```
=> s dimerization
L17     3884 FILE BIOSIS
L18     3216 FILE MEDLINE
L19     3748 FILE EMBASE
L20      165 FILE WPIDS

TOTAL FOR ALL FILES
L21    11013 DIMERIZATION

=> d his

   (FILE 'HOME' ENTERED AT 14:53:29 ON 28 JUN 1997)

   FILE 'BIOSIS, MEDLINE, EMBASE, WPIDS' ENTERED AT 14:53:50
   ON 28 JUN
   1997
L1      1553 FILE BIOSIS
L2       811 FILE MEDLINE
L3       851 FILE EMBASE
L4        67 FILE WPIDS
   TOTAL FOR ALL FILES
L5      3282 S VEGF OR VASCULAR ENDOTHELIAL (1A) GROWTH
FACTOR
L6     11869 FILE BIOSIS
L7      9730 FILE MEDLINE
L8      8822 FILE EMBASE
L9     89179 FILE WPIDS
   TOTAL FOR ALL FILES
L10   119600 S MONOMER
L11        2 FILE BIOSIS
L12        2 FILE MEDLINE
L13        2 FILE EMBASE
L14        1 FILE WPIDS
   TOTAL FOR ALL FILES
L15        7 S L10 AND L5
L16        3 DUPLICATE REMOVE L15 (4 DUPLICATES REMOVED)
L17     3884 FILE BIOSIS
L18     3216 FILE MEDLINE
L19     3748 FILE EMBASE
L20      165 FILE WPIDS
   TOTAL FOR ALL FILES
L21    11013 S DIMERIZATION

=> s l21 and l5
L22        8 FILE BIOSIS
L23        9 FILE MEDLINE
L24        8 FILE EMBASE
L25        0 FILE WPIDS

TOTAL FOR ALL FILES
L26       25 L21 AND L5

=> duplicate remove l26
DUPLICATE PREFERENCE IS 'BIOSIS, MEDLINE, EMBASE'
KEEP DUPLICATES FROM MORE THAN ONE FILE? Y/(N):n
PROCESSING COMPLETED FOR L26
L27       11 DUPLICATE REMOVE L26 (14 DUPLICATES REMOVED)

=> d 1-
```

L27 ANSWER 1 OF 11 BIOSIS COPYRIGHT 1997 BIOSIS
DUPLICATE 1
AN 97:221744 BIOSIS
DN 99513460
TI Mapping of the sites for ligand binding and receptor ***dimerization*** at the extracellular domain of the ***vascular*** ***endothelial*** ***growth*** ***factor*** receptor FLT-1.
AU Barleon B; Totzke F; Herzog C; Blanke S; Kremmer E; Siemeister G; Marme D; Martiny-Baron G
CS Inst. Molecular Med., Tumor Biol. Cent., Breisacher Str. 117, D-79106 Freiburg, Germany
SO Journal of Biological Chemistry 272 (16). 1997. 10382-10388. ISSN: 0021-9258
LA English

L27 ANSWER 2 OF 11 MEDLINE
AN 97272213 MEDLINE
TI A novel bHLH-PAS factor with close sequence similarity to hypoxia-inducible factor 1alpha regulates the ***VEGF*** expression and is potentially involved in lung and vascular development.
AU Ema M; Taya S; Yokotani N; Sogawa K; Matsuda Y; Fujii-Kuriyama Y
CS Department of Chemistry, Graduate School of Science, Tohoku University, Sendai 980-77, Japan.
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1997 Apr 29) 94 (9) 4273-8.
Journal code: PV3. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals

OS GENBANK-D89787
EM 9707
EW 19970705

L27 ANSWER 3 OF 11 BIOSIS COPYRIGHT 1997 BIOSIS
DUPLICATE 2
AN 97:131633 BIOSIS
DN 99423446
TI Structural and functional analysis of hypoxia-inducible factor 1.
AU Semenza G L; Agani F; Booth G; Forsythe J; Iyer N; Jiang B-H; Leung S; Roe R; Wiener C; Yu A
CS Cent. Med. Genet., Johns Hopkins Hosp., CMSC-1004, 600 N. Wolfe St., Baltimore, MD 21287-3914, USA
SO Kidney International 51 (2). 1997. 553-555. ISSN: 0085-2538
LA English

L27 ANSWER 4 OF 11 BIOSIS COPYRIGHT 1997 BIOSIS
AN 97:45582 BIOSIS
DN 99344785
TI Protein tyrosine kinase receptors.
AU Heldin C-H
CS Ludwig Inst. Cancer Res., Box 595, Biomedical Cent., S-751 24 Uppsala, Sweden
SO Parker, P. J. and T. Pawson (Ed.). Cancer Surveys, Vol. 27. Cell signalling. vii+386p. Cold Spring Harbor Laboratory Press: Plainview, New York, USA. 27 (0). 1996. 7-24. ISBN: 0-87969-484-X
DT Book
LA English

L27 ANSWER 5 OF 11 MEDLINE
AN 97117144 MEDLINE
TI In vivo angiogenic activity and hypoxia induction of heterodimers of placenta ***growth*** ***factor*** / ***vascular*** ***endothelial*** ***growth*** ***factor*** .
AU Cao Y; Linden P; Shima D; Browne F; Folkman J
CS Department of Surgery, Harvard Medical School, Boston, Massachusetts 02115, USA.
NC CA-45548 (NCI)
SO JOURNAL OF CLINICAL INVESTIGATION, (1996 Dec 1) 98 (11) 2507-11.
Journal code: HS7. ISSN: 0021-9738.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals; Cancer Journals
EM 9703
EW 19970304

L27 ANSWER 6 OF 11 BIOSIS COPYRIGHT 1997 BIOSIS
DUPLICATE 3
AN 96:363026 BIOSIS
DN 99085382
TI Synthesis and physiological activity of heterodimers comprising different splice forms of ***vascular*** ***endothelial*** ***growth*** ***factor*** and placenta growth factor.
AU Birkenhaeger R; Schneppe B; Roeckl W; Wilting J; Weich H A; McCarthy J E G
CS Dep. Gene Expression, National Biotechnol. Res. Cent., Mascheroder Weg 1, D-38124 Braunschweig, Germany
SO Biochemical Journal 316 (3). 1996. 703-707. ISSN: 0264-6021
LA English

L27 ANSWER 7 OF 11 EMBASE COPYRIGHT 1997 ELSEVIER SCI. B.V.
AN 96304457 EMBASE
TI Identification of a natural soluble form of the ***vascular*** ***endothelial*** ***growth*** ***factor*** receptor, FLT-1, and its heterodimerization with KDR.
AU Kendall R.L.; Wang G.; Thomas K.A.
CS Department of Pharmacology, Merck Research Laboratories, West Point, PA 19486, United States
SO Biochemical and Biophysical Research Communications, (1996) 226/2 (324-328).
ISSN: 0006-291X CODEN: BBRCA
CY United States
DT Journal
FS 029 Clinical Biochemistry
LA English
SL English

L27 ANSWER 8 OF 11 BIOSIS COPYRIGHT 1997 BIOSIS
DUPLICATE 4
AN 95:509281 BIOSIS
DN 98514331
TI Functional interaction of ligands and receptors of the hematopoietic superfamily in yeast.
AU Ozenberger B A; Young K H
CS Agric. Res. Center, American Cyanamid Company, Molecular, Cellular Biol. Group, PO Box 400, Princeton, NJ 08543-0400, USA
SO Molecular Endocrinology 9 (10). 1995. 1321-1329. ISSN: 0888-8809
LA English

L27 ANSWER 9 OF 11 BIOSIS COPYRIGHT 1997 BIOSIS
DUPLICATE 5
AN 95:103809 BIOSIS
DN 98118109
TI Structural requirements for ***dimerization*** , glycosylation, secretion, and biological function of VPF- ***VEGF*** .
AU Claffey K P; Senger D R; Spiegelman B M
CS Dep. Pathology, Beth Israel Hosp., Harvard Med. Sch., Boston, MA 02215, USA
SO Biochimica et Biophysica Acta 1246 (1). 1995. 1-9. ISSN: 0006-3002
LA English

L27 ANSWER 10 OF 11 BIOSIS COPYRIGHT 1997 BIOSIS
DUPLICATE 6
AN 95:106019 BIOSIS
DN 98120319
TI Covalent ***Dimerization*** of Vascular Permeability Factor- ***Vascular*** ***Endothelial*** ***Growth*** ***Factor*** Is Essential for Its Biological Activity: Evidence from Cys to Ser mutations.
AU Potgens A J G; Lubsen N H; Van Altena M C; Vermeulen R; Bakker A; Schoenmakers J G G; Ruiter D J; De Waal R M W
CS Inst. Pathol., Univ. Hosp. Njimegen, P. O. Box 9101, NL-6500 HB Nijmegen, Netherlands
SO Journal of Biological Chemistry 269 (52). 1994. 32879-32885. ISSN: 0021-9258
LA English

L27 ANSWER 11 OF 11 BIOSIS COPYRIGHT 1997 BIOSIS
DUPLICATE 7
AN 93:209115 BIOSIS
DN BA95:110340
TI ALANINE MUTAGENESIS OF CONSERVED RESIDUES IN THE PLATELET-DERIVED GROWTH FACTOR FAMILY IDENTIFICATION OF RESIDUES NECESSARY FOR ***DIMERIZATION*** AND TRANSFORMATION.
AU MAHER D W; STRAWN L M; DONOGHUE D J
CS DEP. CHEM./DIV. BIOCHEM., CENTER MOL. GENETICS, UNIVERSITY CALIFORNIA SAN DIEGO, LA JOLLA, CA 92093-0322, USA.
SO ONCOGENE 8 (3). 1993. 533-541. CODEN: ONCNES ISSN: 0950-9232
LA English

=> d 11 abs

L27 ANSWER 11 OF 11 BIOSIS COPYRIGHT 1997 BIOSIS
DUPLICATE 7
AN 93:209115 BIOSIS
AB Platelet-derived growth factor (PDGF) and vascular endothelial factor define a family of dimeric proteins characterized by eight conserved cysteine residues involved in disulfide bonds. Thirteen non-cysteine residues conserved among the platelet-derived/vascular endothelial growth factors were individually mutated to alanine in v-sis/PDGF-B. In addition, five other residues flanking F148 were also mutated to alanine. The resulting mutants were assayed for transformation of NIH3T3 cells, and the mutant proteins were assayed for their ability to dimerize. Four residues were found to be crucial for disulfide-linked dimer formation: P152 and G162 were mandatory, while R159 and H205 also contributed to efficient ***dimerization*** . Four of the mutant proteins (at residues N147, F148, L149 and K185) dimerized efficiently yet exhibited less than 50% transforming activity compared with wild-type v-sis. Two mutants (at residues D142 and F148) were located in a region important for PDGF receptor

interaction and were further studied with regard to secretion and PDGF receptor autophosphorylation. A series of substituions at residue F148 revealed a strong preference for aromatic amino acids. One mutant from this series (F148G) dimerized but was completely inactive for transformation. This study identifies four residues in v-sis/PDGF-B important for ***dimerization*** and also identifies additional residues critical for full activation of PDGF receptors. The E5 oncoprotein encoded by bovine papillomavirus type I exhibits two short regions of amino acid similarity when compared with the minimal transforming region of v-sis/PDGF-B. Several of the v-sis mutants discussed in this work affect residues that are also present in the E5 oncoprotein, including F148, L149 and H205.

=> d 10 abs

L27 ANSWER 10 OF 11 BIOSIS COPYRIGHT 1997 BIOSIS
DUPLICATE 6
AN 95:106019 BIOSIS
AB Vascular permeability factor, or ***vascular*** ***endothelial*** ***growth*** ***factor*** (VPF/ ***VEGF***) is an important factor in the regulation of vascular growth and vascular permeability. VPF is a secreted, dimeric protein and has 8 cysteine residues conserved with platelet-derived growth factor (PDGF). To study the role of some of these cysteine residues in maintaining the structure and function of VPF, we replaced the codons for the second, third, fourth, and fifth cysteine by serine codons, and expressed the mutant proteins in a mammalian expression system. Cysteine residues 2 and 4 in VPF were found to be directly involved in anti-parallel interchain disulfide bonds, as in PDGF. VPF mutants lacking one of these cysteines were severely impaired in their S-linked ***dimerization*** , while upon coexpression of both mutants the ability to form dimers was restored. The VPF mutants lacking cysteine residue 2 or 4 also competed poorly for receptor binding of labeled VPF and had low biological activity, but these defects were also complemented by coexpressing the two mutants, indicating that for efficient receptor binding and activation VPF needs to be a covalent dimer, unlike PDGF-BB. Furthermore, cysteine residue 5 was found to be essential for VPF ***dimerization*** and activity, while the mutant lacking cysteine residue 3 was only mildly affected in its ability to dimerize and had partial biological activity.

=> s cys or cysteine
L28 36793 FILE BIOSIS
L29 33747 FILE MEDLINE
L30 26488 FILE EMBASE
L31 5023 FILE WPIDS

TOTAL FOR ALL FILES
L32 102051 CYS OR CYSTEINE

=> s 51 or 60
L33 288872 FILE BIOSIS
L34 252274 FILE MEDLINE
L35 227825 FILE EMBASE
L36 289801 FILE WPIDS

TOTAL FOR ALL FILES
L37 1058772 51 OR 60

=> s l37 and l32
L38 1807 FILE BIOSIS
L39 1564 FILE MEDLINE
L40 1293 FILE EMBASE
L41 338 FILE WPIDS

TOTAL FOR ALL FILES
L42 5002 L37 AND L32

=> s l42 and l5
L43 1 FILE BIOSIS
L44 1 FILE MEDLINE
L45 0 FILE EMBASE
L46 0 FILE WPIDS

TOTAL FOR ALL FILES
L47 2 L42 AND L5

=> duplicate remove l47
DUPLICATE PREFERENCE IS 'BIOSIS, MEDLINE'
KEEP DUPLICATES FROM MORE THAN ONE FILE? Y/(N):n
PROCESSING COMPLETED FOR L47
L48 1 DUPLICATE REMOVE L47 (1 DUPLICATE REMOVED)

=> d

L48 ANSWER 1 OF 1 BIOSIS COPYRIGHT 1997 BIOSIS
DUPLICATE 1
AN 97:13697 BIOSIS
DN 99312900
TI Crystallization of the receptor binding domain of ***vascular*** ***endothelial*** ***growth*** ***factor*** .
AU Christinger H W; Muller Y A; Berleau L T; Keyt B A; Cunningham B C; Ferrara N; De Vos A M
CS Dep. Protein Eng., Genentech Inc., 460 Point San Bruno Blvd., South San Francisco, CA 94080, USA
SO Proteins Structure Function and Genetics 26 (3). 1996. 353-357. ISSN: 0887-3585
LA English

=> d abs

L48 ANSWER 1 OF 1 BIOSIS COPYRIGHT 1997 BIOSIS
DUPLICATE 1
AN 97:13697 BIOSIS
AB ***Vascular*** ***endothelial*** ***growth*** ***factor*** (***VEGF***) is a potent angiogenic factor with a unique specificity for vascular endothelial cells. In addition to its role in vasculogenesis and embryonic angiogenesis, ***VEGF*** is implicated in pathologic neovascularization associated with tumors and diabetic retinopathy. Four different constructs of a short variant of ***VEGF*** sufficient for receptor binding were overexpressed in Escherichia coli, refolded, purified, and crystallized in five different space groups. In order to facilitate the production of heavy atom derivatives, single ***cysteine*** mutants were designed based on the crystal structure of platelet-derived growth factor. A construct consisting of residues 8 to 109 was crystallized in space group P2-1, with cell parameters a = 55.6 ANG , b = ***60*** .4 ANG , c = 77.7 ANG , beta = 90.0 degree , and four monomers in the asymmetric unit. Native and derivative data were collected for two of the ***cysteine*** mutants as well as for wild-type ***VEGF*** .

=> s l32 and l5
L49 10 FILE BIOSIS
L50 11 FILE MEDLINE
L51 10 FILE EMBASE
L52 4 FILE WPIDS

TOTAL FOR ALL FILES
L53 35 L32 AND L5

=> duplicate remove l53
DUPLICATE PREFERENCE IS 'BIOSIS, MEDLINE, EMBASE, WPIDS'
KEEP DUPLICATES FROM MORE THAN ONE FILE? Y/(N):n
PROCESSING COMPLETED FOR L53
L54 17 DUPLICATE REMOVE L53 (18 DUPLICATES REMOVED)

=> d l-

L54 ANSWER 1 OF 17 WPIDS COPYRIGHT 1997 DERWENT INFORMATION LTD
AN 97-099920 [09] WPIDS
DNC C97-031900
TI Activating cell surface receptors using peptide dimer agonists - also, new dimers of erythropoietin receptor binding peptide(s) useful for treating patient having disorder characterised by EPO deficiency.
DC B04
IN JOHNSON, D L; ZIVIN, R A
PA (ORTH) ORTHO PHARM CORP; (JOHJ) JOHNSON & JOHNSON
CYC 70
PI WO 9640772 A2 961219 (9709)* EN 110 pp C07K014-505
RW: AT BE CH DE DK EA ES FI FR GB GR IE IT KE LS LU MC MW NL OA
PT SD SE SZ UG
W: AL AM AT AU AZ BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE

HU IS JP KE KG KP KR KZ LK LR LS LT LU LV MD MG MK MN
MW MX
NO NZ PL PT RO RU SD SE SG SI SK TJ TM TR TT UA UG US
UZ VN
AU 9661007 A 961230 (9716) C07K014-505
ADT WO 9640772 A2 WO 96-US9469 960606; AU 9661007 A AU 96-61007
960606
FDT AU 9661007 A Based on WO 9640772
PRAI US 95-484135 950607
IC ICM C07K014-505
ICS A61K038-18

L54 ANSWER 2 OF 17 WPIDS COPYRIGHT 1997 DERWENT
INFORMATION LTD
AN 96-160151 [16] WPIDS
CR 96-179728 [18]; 97-011855 [01]
DNC C96-050536
TI ***Vascular*** ***endothelial*** cell ***growth***
factor (***VEGF***) conjugates - having ***VEGF***
linked to targetted agent, used for inhibiting proliferation of
cells, e.g. for gene therapy.
DC B04 D16
IN FLEURBAAIJ, G A; FREUND, E; HOUSTON, L L; NOVA, M P;
SOSNOWSKI, B A;
VICTOR, K D
PA (PRIZ-N) PRIZM PHARM INC
CYC 63
PI WO 9606641 A1 960307 (9616)* EN 193 pp A61K047-48
RW: AT BE CH DE DK ES FR GB GR IE IT KE LU MC MW NL OA
PT SD SE
SZ UG
W: AM AU BB BG BR BY CA CN CZ EE FI GE HU IS JP KG KP KR
KZ LK
LR LT LV MD MG MN MW MX NO NZ PL RO RU SG SI SK TJ
TM TT UA
UZ VN
AU 9533747 A 960322 (9626) A61K047-48
ADT WO 9606641 A1 WO 95-US10973 950829; AU 9533747 A AU
95-33747 950829
FDT AU 9533747 A Based on WO 9606641
PRAI US 95-441979 950516; US 94-297961 940829
IC ICM A61K047-48
ICS A61K041-00; C07K014-475; C07K019-00; C12N001-21;
C12N015-12;
C12N015-62

L54 ANSWER 3 OF 17 BIOSIS COPYRIGHT 1997 BIOSIS
DUPLICATE 1
AN 97:200741 BIOSIS
DN 99499944
TI Primary culture and characterization of microvascular endothelial
cells from Macaca monkey retina.
AU Yan Q; Vernon R B; Hendrickson A E; Sage E H
CS Dep. Biological Structure, Box 357420, Univ. Washington, Seattle, WA
98195-7420, USA
SO Investigative Ophthalmology & Visual Science 37 (11). 1996.
2185-2194. ISSN: 0146-0404
LA English

L54 ANSWER 4 OF 17 BIOSIS COPYRIGHT 1997 BIOSIS
DUPLICATE 2
AN 96:183565 BIOSIS
DN 98739694
TI ***Vascular*** ***endothelial*** ***growth***
factor -related protein: A ligand and specific activator of
the tyrosine kinase receptor Flt4.
AU Lee J; Gray A; Yuan J; Luoh S-M; Avraham H; Wood W I
CS Dep. Mol. Biol., Genentech, Inc., 460 Point San Bruno Boulevard,
South San Francisco, CA 94080, USA
SO Proceedings of the National Academy of Sciences of the United States
of America 93 (5). 1996. 1988-1992. ISSN: 0027-8424
LA English

L54 ANSWER 5 OF 17 BIOSIS COPYRIGHT 1997 BIOSIS
AN 97:96708 BIOSIS
DN 99395911
TI SPARC inhibits ***vascular*** ***endothelial*** cell
growth ***factor*** (***VEGF***)-stimulated
proliferation of human microvascular endothelial cells (HMVEC) by
direct interaction with ***VEGF*** .
AU Kupprion C; Sage E H
CS Dep. Biol. Structure, Univ. Washington, Seattle, WA 98195, USA
SO Annual Meeting of the 6th International Congress on Cell Biology and
the 36th American Society for Cell Biology, San Francisco,
California, USA, December 7-11, 1996. Molecular Biology of the Cell 7
(SUPPL.). 1996. 414A. ISSN: 1059-1524
DT Conference
LA English

L54 ANSWER 6 OF 17 BIOSIS COPYRIGHT 1997 BIOSIS
DUPLICATE 3
AN 97:13697 BIOSIS
DN 99312900
TI Crystallization of the receptor binding domain of ***vascular***
endothelial ***growth*** ***factor*** .
AU Christinger H W; Muller Y A; Berleau L T; Keyt B A; Cunningham B C;
Ferrara N; De Vos A M
CS Dep. Protein Eng., Genentech Inc., 460 Point San Bruno Blvd., South
San Francisco, CA 94080, USA
SO Proteins Structure Function and Genetics 26 (3). 1996. 353-357.
ISSN: 0887-3585
LA English

L54 ANSWER 7 OF 17 BIOSIS COPYRIGHT 1997 BIOSIS
DUPLICATE 4
AN 96:121582 BIOSIS
DN 98693717
TI A novel ***vascular*** ***endothelial*** ***growth***
factor , ***VEGF*** -C, is a ligand for the Flt4 (VEGFR-3)
and KDR (VEGFR-2) receptor tyrosine kinase.
AU Joukouv V; Pajusola K; Kaipainen A; Chilov D; Lahtinen I; Kukk E;
Saksela O; Kalkkinen N; Alitalo K
CS Molecular/Cancer Biol. Lab., Haartman Inst., Univ. Helsinki, PL21
Haartmanink 3, 00014 Helsinki, Finland
SO EMBO (European Molecular Biology Organization) Journal 15 (2). 1996.
290-298. ISSN: 0261-4189
LA English

L54 ANSWER 8 OF 17 MEDLINE DUPLICATE 5
AN 97077124 MEDLINE
TI Cloning and characterization of a novel human gene related to
vascular ***endothelial*** ***growth***
factor .
AU Grimmond S; Lagercrantz J; Drinkwater C; Silins G; Townson S;
Pollock P; Gotley D; Carson E; Rakar S; Nordenskjold M; Ward L;
Hayward N; Weber G
CS Queensland Cancer Fund Research Unit Joint Experimental Oncology
Program, Queensland Institute of Medical Research, Herston,
Australia.. seanG@qimr.edu.au
SO GENOME RESEARCH, (1996 Feb) 6 (2) 124-31.
Journal code: CES. ISSN: 1088-9051.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 9702
EW 19970204

L54 ANSWER 9 OF 17 BIOSIS COPYRIGHT 1997 BIOSIS
DUPLICATE 6
AN 96:380088 BIOSIS
DN 99102444
TI The placenta growth factor gene of the mouse
AU Dipalma T; Tucci M; Russo G; Maglione D; Lago C T; Romano A,
Saccone
S; Della Valle G; De Gregorio L; Dragnani T A; Viglietto G; Persico M
G
CS Int. Inst. Genetics Biophysics, CNR, Via Marconi 10, 80125 Naples,
Italy
SO Mammalian Genome 7 (1). 1996. 6-12. ISSN: 0938-8990
LA English

L54 ANSWER 10 OF 17 BIOSIS COPYRIGHT 1997 BIOSIS
DUPLICATE 7
AN 95:103809 BIOSIS
DN 98118109
TI Structural requirements for dimerization, glycosylation, secretion,
and biological function of VPF- ***VEGF*** .

AU Claffey K P; Senger D R; Spiegelman B M
CS Dep. Pathology, Beth Israel Hosp., Harvard Med. Sch., Boston, MA 02215, USA
SO Biochimica et Biophysica Acta 1246 (1). 1995. 1-9. ISSN: 0006-3002
LA English

L54 ANSWER 11 OF 17 BIOSIS COPYRIGHT 1997 BIOSIS
DUPLICATE 8
AN 95:106019 BIOSIS
DN 98120319
TI Covalent Dimerization of Vascular Permeability Factor- ***Vascular*** ***Endothelial*** ***Growth*** ***Factor*** Is Essential for Its Biological Activity: Evidence from ***Cys*** to Ser mutations.
AU Potgens A J G; Lubsen N H; Van Altena M C; Vermeulen R; Bakker A; Schoenmakers J G G; Ruiter D J; De Waal R M W
CS Inst. Pathol., Univ. Hosp. Njimegen, P. O. Box 9101, NL-6500 HB Nijmegen, Netherlands
SO Journal of Biological Chemistry 269 (52). 1994. 32879-32885. ISSN: 0021-9258
LA English

L54 ANSWER 12 OF 17 EMBASE COPYRIGHT 1997 ELSEVIER SCI. B.V.
AN 93107814 EMBASE
TI Identification and localization of alternately spliced mRNAs for ***vascular*** ***endothelial*** ***growth*** ***factor*** in human uterus and estrogen regulation in endometrial carcinoma cell lines.
AU Charnock-Jones D.S.; Sharkey A.M.; Rajput-Williams J.; Burch D.; Schofield J.P.; Fountain S.A.; Boocock C.A.; Smith S.K.
CS Department of Obstetrics/Gynaecology, Rosie Maternity Hospital, University of Cambridge, Robinson Way, Cambridge CB2 2SW, United Kingdom
SO BIOL. REPROD., (1993) 48/5 (1120-1128).
ISSN: 0006-3363 CODEN: BIREBV
CY United States
DT Journal
FS 010 Obstetrics and Gynecology
022 Human Genetics
LA English
SL English

L54 ANSWER 13 OF 17 BIOSIS COPYRIGHT 1997 BIOSIS
DUPLICATE 9
AN 93:209115 BIOSIS
DN BA95:110340
TI ALANINE MUTAGENESIS OF CONSERVED RESIDUES IN THE PLATELET-DERIVED
GROWTH FACTOR FAMILY IDENTIFICATION OF RESIDUES NECESSARY FOR
DIMERIZATION AND TRANSFORMATION.
AU MAHER D W; STRAWN L M; DONOGHUE D J
CS DEP. CHEM./DIV. BIOCHEM., CENTER MOL. GENETICS, UNIVERSITY CALIFORNIA
SAN DIEGO, LA JOLLA, CA 92093-0322, USA.
SO ONCOGENE 8 (3). 1993. 533-541. CODEN: ONCNES ISSN: 0950-9232
LA English

L54 ANSWER 14 OF 17 MEDLINE
AN 93100326 MEDLINE
TI Dual regulation of ***vascular*** ***endothelial*** ***growth*** ***factor*** bioavailability by genetic and proteolytic mechanisms.
AU Houck K A; Leung D W; Rowland A M; Winer J; Ferrara N
CS Department of Molecular Biology, Genetech, Inc., South San Francisco, California 94080..
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1992 Dec 25) 267 (36) 26031-7.
Journal code: HIV. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 9303

L54 ANSWER 15 OF 17 WPIDS COPYRIGHT 1997 DERWENT INFORMATION LTD
AN 91-073534 [10] WPIDS
DNC C91-031171
TI DNA encoding ***vascular*** ***endothelial*** cell ***growth*** ***factor*** - used for producing the factor for angiogenesis and re-endothelialisation in wound healing.
DC B04 D16
IN ABRAHAM, J A; FIDDES, J C; MITCHELL, R L; TISCHER, E G
PA (CALD) CALIFORNIA BIOTECHNOLOGY INC; (SCIO-N) SCIOS NOVA INC
CYC 16
PI WO 9102058 A 910221 (9110)*
AU 9060798 A 910311 (9123)
EP 484401 A 920513 (9220) EN 94 pp
US 5194596 A 930316 (9313) C07K003-00
JP 05501350 W 930318 (9316) 34 pp C12N015-18
EP 484401 A4 920819 (9523)
EP 484401 B1 960911 (9641) EN 59 pp C12N015-12
R: AT BE CH DE DK ES FR GB IT LI LU NL SE
DE 69028535 E 961017 (9647) C12N015-12
ES 2094159 T3 970116 (9710) C12N015-12
ADT EP 484401 A EP 90-911525 900727; US 5194596 A CIP of US 89-387545
890727, US 89-450883 891214; JP 05501350 W JP 90-511123 900727, WO 90-US4227 900727; EP 484401 A4 EP 90-911525 ; EP 484401 B1 EP 90-911525 900727, WO 90-US4227 900727; DE 69028535 E DE 90-628535
900727, EP 90-911525 900727, WO 90-US4227 900727; ES 2094159 T3 EP
90-911525 900727
FDT JP 05501350 W Based on WO 9102058; EP 484401 B1 Based on WO 9102058;
DE 69028535 E Based on EP 484401, Based on WO 9102058; ES 2094159 T3
Based on EP 484401
PRAI US 89-450883 891214; US 89-387545 890727
IC ICM C07K003-00; C12N015-12; C12N015-18
ICS A61K037-36; A61K038-27; C12N005-10; C12N015-00

L54 ANSWER 16 OF 17 WPIDS COPYRIGHT 1997 DERWENT INFORMATION LTD
AN 91-003167 [12] WPIDS
DNC C91-001433
TI ***Vascular*** ***endothelial*** cell ***growth*** ***factor*** - prepd. by culturing human placenta in serum-free medium.
DC B04
PA (MKME-N) YG M K MEDICAL
CYC 1
PI JP 02279698 A 901115 (9312)*
ADT JP 02279698 A JP 89-98931 890420
PRAI JP 89-98931 890420
IC A61K037-02; C07K003-18; C07K015-12

L54 ANSWER 17 OF 17 BIOSIS COPYRIGHT 1997 BIOSIS
DUPLICATE 10
AN 90:88756 BIOSIS
DN BA89:48107
TI ***VASCULAR*** ***ENDOTHELIAL*** ***GROWTH*** ***FACTOR*** A NEW MEMBER OF THE PLATELET-DERIVED GROWTH FACTOR
GENE FAMILY.
AU TISCHER E; GOSPODAROWICZ D; MITCHELL R; SILVA M; SCHILLING J; LAU K;
CRISP T; FIDDES J C; ABRAHAM J A
CS CALIF. BIOTECHNOL. INC., 2450 BAYSHORE PARKWAY, MOUNTAIN VIEW, CALIF.
94043.
SO BIOCHEM BIOPHYS RES COMMUN 165 (3). 1989. 1198-1206. CODEN: BBRCA9
ISSN: 0006-291X
LA English

=> d 10 abs

L54 ANSWER 10 OF 17 BIOSIS COPYRIGHT 1997 BIOSIS
DUPLICATE 7
AN 95:103809 BIOSIS
AB Vascular permeability factor (VPF) also known as ***vascular*** ***endothelial*** ***growth*** ***factor*** (***VEGF***

), is a dimeric protein that affects endothelial cell (EC) and vascular functions including enhancement of microvascular permeability and stimulation of EC growth. To investigate the structural features of VPF/ ***VEGF*** necessary for efficient dimerization, secretion, and biological activities, we employed site-directed mutagenesis with a Cos-1 cell expression system. Several ***cysteine*** residues essential for VPF dimerization were identified by mutation analysis of the ***Cys*** -25, ***Cys*** -56, and ***Cys*** -67 residues. Mutant VPF isoforms lacking either of these cysteines were secreted as monomers and were completely inactive in both vascular permeability and endothelial cell mitotic assays. VPF ***Cys*** -145 mutant protein was efficiently secreted as a glycosylated, dimeric polypeptide, but had a reduction in biological activities. The site of N-linked glycosylation was directly identified as Asn-74, which, when mutated produced an inefficiently secreted dimeric protein without post-translational glycosylation, yet maintained full vascular permeability activity. Finally, we found that one VPF mutant isoform ***Cys*** -101 was not secreted and this mutant functioned as a dominant-negative suppressor of wild-type VPF secretion as demonstrated by co-expression assays in Cos-1 cells.

=> d 10-11

L54 ANSWER 10 OF 17 BIOSIS COPYRIGHT 1997 BIOSIS
DUPLICATE 7
AN 95:103809 BIOSIS
DN 98118109
TI Structural requirements for dimerization, glycosylation, secretion, and biological function of VPF- ***VEGF*** .
AU Claffey K P; Senger D R; Spiegelman B M
CS Dep. Pathology, Beth Israel Hosp., Harvard Med. Sch., Boston, MA 02215, USA
SO Biochimica et Biophysica Acta 1246 (1). 1995. 1-9. ISSN: 0006-3002
LA English

L54 ANSWER 11 OF 17 BIOSIS COPYRIGHT 1997 BIOSIS
DUPLICATE 8
AN 95:106019 BIOSIS
DN 98120319
TI Covalent Dimerization of Vascular Permeability Factor- ***Vascular*** ***Endothelial*** ***Growth*** ***Factor*** Is Essential for Its Biological Activity: Evidence from ***Cys*** to Ser mutations.
AU Potgens A J G; Lubsen N H; Van Altena M C; Vermeulen R; Bakker A; Schoenmakers J G G; Ruiter D J; De Waal R M W
CS Inst. Pathol., Univ. Hosp. Njimegen, P. O. Box 9101, NL-6500 HB Nijmegen, Netherlands
SO Journal of Biological Chemistry 269 (52). 1994. 32879-32885. ISSN: 0021-9258
LA English

=> d his

(FILE 'HOME' ENTERED AT 14:53:29 ON 28 JUN 1997)

FILE 'BIOSIS, MEDLINE, EMBASE, WPIDS' ENTERED AT 14:53:50 ON 28 JUN 1997

L1 1553 FILE BIOSIS
L2 811 FILE MEDLINE
L3 851 FILE EMBASE
L4 67 FILE WPIDS
TOTAL FOR ALL FILES
L5 3282 S VEGF OR VASCULAR ENDOTHELIAL (1A) GROWTH FACTOR
L6 11869 FILE BIOSIS
L7 9730 FILE MEDLINE
L8 8822 FILE EMBASE
L9 89179 FILE WPIDS
TOTAL FOR ALL FILES
L10 119600 S MONOMER
L11 2 FILE BIOSIS
L12 2 FILE MEDLINE
L13 2 FILE EMBASE
L14 1 FILE WPIDS
TOTAL FOR ALL FILES
L15 7 S L10 AND L5
L16 3 DUPLICATE REMOVE L15 (4 DUPLICATES REMOVED)
L17 3884 FILE BIOSIS
L18 3216 FILE MEDLINE
L19 3748 FILE EMBASE
L20 165 FILE WPIDS
TOTAL FOR ALL FILES
L21 11013 S DIMERIZATION
L22 8 FILE BIOSIS
L23 9 FILE MEDLINE
L24 8 FILE EMBASE
L25 0 FILE WPIDS
TOTAL FOR ALL FILES
L26 25 S L21 AND L5
L27 11 DUPLICATE REMOVE L26 (14 DUPLICATES REMOVED)
L28 36793 FILE BIOSIS
L29 33747 FILE MEDLINE
L30 26488 FILE EMBASE
L31 5023 FILE WPIDS
TOTAL FOR ALL FILES
L32 102051 S CYS OR CYSTEINE
L33 288872 FILE BIOSIS
L34 252274 FILE MEDLINE
L35 227825 FILE EMBASE
L36 289801 FILE WPIDS
TOTAL FOR ALL FILES
L37 1058772 S 51 OR 60
L38 1807 FILE BIOSIS
L39 1564 FILE MEDLINE
L40 1293 FILE EMBASE
L41 338 FILE WPIDS
TOTAL FOR ALL FILES
L42 5002 S L37 AND L32
L43 1 FILE BIOSIS
L44 1 FILE MEDLINE
L45 0 FILE EMBASE
L46 0 FILE WPIDS
TOTAL FOR ALL FILES
L47 2 S L42 AND L5
L48 1 DUPLICATE REMOVE L47 (1 DUPLICATE REMOVED)
L49 10 FILE BIOSIS
L50 11 FILE MEDLINE
L51 10 FILE EMBASE
L52 4 FILE WPIDS
TOTAL FOR ALL FILES
L53 35 S L32 AND L5
L54 17 DUPLICATE REMOVE L53 (18 DUPLICATES REMOVED)

=> log y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	65.31	65.46

STN INTERNATIONAL LOGOFF AT 15:05:27 ON 28 JUN 1997

FILE 'USPAT' ENTERED AT 15:08:40 ON 28 JUN 1997

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**********************************
*        WELCOME TO THE          *
*     U.S. PATENT TEXT FILE      *
**********************************
```

```
=> s platelet derived growth factor or vascular endothelial (1a) growth factor or
pdgf or vegf
     10473 PLATELET
    284391 DERIVED
    123016 GROWTH
    228048 FACTOR
      1005 PLATELET DERIVED GROWTH FACTOR
             (PLATELET(W)DERIVED(W)GROWTH(W)FACTOR)
     15211 VASCULAR
      3724 ENDOTHELIAL
       510 VASCULAR ENDOTHELIAL
             (VASCULAR(W)ENDOTHELIAL)
    123016 GROWTH
    228048 FACTOR
      4420 GROWTH FACTOR
             (GROWTH(W)FACTOR)
        64 VASCULAR ENDOTHELIAL (1A) GROWTH FACTOR
       790 PDGF
        32 VEGF
L1    1221 PLATELET DERIVED GROWTH FACTOR OR VASCULAR
ENDOTHELIAL (1A)
 GR
        OWTH FACTOR OR PDGF OR VEGF

=> s cysteine?
L2    9928 CYSTEINE?

=> s dimerization or dimer or monomer or heterodimer
      3889 DIMERIZATION
     14150 DIMER
     74366 MONOMER
       399 HETERODIMER
L3   84627 DIMERIZATION OR DIMER OR MONOMER OR
HETERODIMER

=> s l1 and l2 and l3
L4     137 L1 AND L2 AND L3

=> s antagon?
L5   16494 ANTAGON?

=> s l4 and l5
L6      55 L4 AND L5

=> d 1-
```

1. 5,635,388, Jun. 3, 1997, Agonist antibodies against the flk2/flt3 receptor and uses thereof; Brian D. Bennett, et al., 435/334; 424/85.1, 85.2, 85.5; 435/70.21, 172.2, 320.1, 328; 530/351, 387.3, 388.22, 389.1; 536/23.53 [IMAGE AVAILABLE]

2. 5,635,177, Jun. 3, 1997, Protein tyrosine kinase agonist antibodies; Brian D. Bennett, et al., 424/143.1, 138.1, 146.1, 155.1; 435/330, 334, 338; 530/387.7, 388.26 [IMAGE AVAILABLE]

3. 5,627,073, May 6, 1997, Hybridomas producing antibodies to cardiac hypertrophy factor; Joffre Baker, et al., 435/331; 424/139.1, 145.1; 435/69.6, 70.21, 172.1, 252.33, 332, 336; 530/387.3, 387.9, 388.23, 391.3 [IMAGE AVAILABLE]

4. 5,624,818, Apr. 29, 1997, Nucleic acids encoding regulatory proteins that dimerize with Mad or Max; Robert N. Eisenman, et al., 435/69.1, 70.1, 172.3, 252.3, 320.1; 536/23.1, 23.5; 935/11, 22, 70, 72 [IMAGE AVAILABLE]

5. 5,624,816, Apr. 29, 1997, Transmembrane glycoprotein ASGP-2: nucleotide sequences and recombinant production of proteins; Kermit L. Carraway, et al., 435/69.1, 69.3, 172.1, 252.3, 320.1, 325; 530/395; 536/23.5 [IMAGE AVAILABLE]

6. 5,624,806, Apr. 29, 1997, Antibodies to cardiac hypertrophy factor and uses thereof; Joffre Baker, et al., 435/7.1; 530/387.3, 387.9, 388.85, 391.3 [IMAGE AVAILABLE]

7. 5,621,081, Apr. 15, 1997, Glial mitogenic factors; Andrew Goodearl, et al., 530/350, 395, 399 [IMAGE AVAILABLE]

8. 5,616,311, Apr. 1, 1997, Non-crosslinked protein particles for therapeutic and diagnostic use; Richard C. K. Yen, 424/1.33, 1.29, 1.37, 484, 499; 427/2.14, 2.21, 213.3, 213.33; 428/402.2, 402.24; 435/177; 935/54 [IMAGE AVAILABLE]

9. 5,607,918, Mar. 4, 1997, **Vascular** **endothelial** **growth** **factor**-B and DNA coding therefor; Ulf Eriksson, et al., 514/12; 530/350 [IMAGE AVAILABLE]

10. 5,606,032, Feb. 25, 1997, Process for preparing glial mitogenic factors; Andrew Goodearl, et al., 530/416, 413, 414, 415, 417 [IMAGE AVAILABLE]

11. 5,602,096, Feb. 11, 1997, Method of using a secretable glial mitogenic factor to induce acetylcholine receptor synthesis; Andrew Goodearl, et al., 514/12; 435/69.1; 514/2; 530/350 [IMAGE AVAILABLE]

12. 5,594,114, Jan. 14, 1997, Schwann cell mitogenic factor, its preparation and use; Andrew D. J. Goodearl, et al., 530/399 [IMAGE AVAILABLE]

13. 5,585,270, Dec. 17, 1996, Polynucleotides encoding connective tissue growth factor; Gary R. Grotendorst, et al., 435/252.3; 424/198.1; 435/320.1, 325, 348; 530/399; 536/23.5 [IMAGE AVAILABLE]

14. 5,580,963, Dec. 3, 1996, Single-chain hepatocyte growth factor variants; Paul J. Godowski, et al., 530/399 [IMAGE AVAILABLE]

15. 5,578,482, Nov. 26, 1996, Ligand growth factors that bind to the erbB-2 receptor protein and induce cellular responses; Marc E. Lippman, et al., 435/384, 244, 377, 387; 514/21; 530/350, 399 [IMAGE AVAILABLE]

16. 5,573,762, Nov. 12, 1996, Use of leukemia inhibitory factor specific antibodies and endothelin **antagonists** for treatment of cardiac hypertrophy; Napoleone Ferrara, et al., 424/145.1; 435/70.21, 172.2; 514/11, 17; 530/300, 388.23, 388.24 [IMAGE AVAILABLE]

17. 5,571,893, Nov. 5, 1996, Cardiac hypertrophy factor; Joffre Baker, et al., 530/350, 351, 399; 930/140 [IMAGE AVAILABLE]

18. 5,571,675, Nov. 5, 1996, Detection and amplification of candiotrophin-1(cardiac hypertrophy factor); Joffre Baker, et al., 435/6, 91.2, 91.21; 536/24.3, 24.31, 24.32, 24.33 [IMAGE AVAILABLE]

19. 5,567,584, Oct. 22, 1996, Methods of using biologically active dimerized polypeptide fusions to detect **PDGF**; Andrzej Z. Sledziewski, et al., 435/6, 7.1, 69.7; 436/501; 536/23.4 [IMAGE AVAILABLE]

20. 5,547,856, Aug. 20, 1996, Hepatocyte growth factor variants; Paul J. Godowski, et al., 435/69.4, 320.1, 325; 530/399; 536/23.51 [IMAGE AVAILABLE]

21. 5,534,615, Jul. 9, 1996, Cardiac hypertrophy factor and uses therefor; Joffre Baker, et al., 530/350; 424/569, 570; 530/380 [IMAGE AVAILABLE]

22. 5,530,109, Jun. 25, 1996, DNA encoding glial mitogenic factors; Andrew Goodearl, et al., 536/23.5; 435/252.3, 320.1; 530/399 [IMAGE AVAILABLE]

23. 5,521,073, May 28, 1996, TIE-2 ligand, and method of making; Samuel Davis, et al., 435/69.5, 70.1, 252.3, 320.1, 348, 365; 536/23.1, 23.5, 24.3, 24.31; 935/22, 69, 70, 72 [IMAGE AVAILABLE]

24. 5,514,582, May 7, 1996, Recombinant DNA encoding hybrid immunoglobulins; Daniel J. Capon, et al., 435/252.3, 69.7, 320.1; 536/23.5, 23.52, 23.53 [IMAGE AVAILABLE]

25. 5,512,545, Apr. 30, 1996, **PDGF**-B analogues; David Brown, et al., 514/12; 435/69.4, 252.33, 255.1, 320.1; 530/350, 399; 536/23.51 [IMAGE AVAILABLE]

26. 5,506,107, Apr. 9, 1996, Selecting ligand agonists and **antagonists**; Brian C. Cunningham, et al., 435/7.21, 7.8; 436/501, 537

[IMAGE AVAILABLE]

27. 5,504,197, Apr. 2, 1996, DNA encoding neurotrophic growth factors; David R. Schubert, et al., 536/23.5; 435/69.1, 252.33, 320.1; 935/23, 66 [IMAGE AVAILABLE]

28. 5,488,099, Jan. 30, 1996, Multifunctional chimeric neurotrophic factors; Hakan B. Persson, deceased, et al., 530/399; 435/69.1, 320.1; 536/23.5 [IMAGE AVAILABLE]

29. 5,486,599, Jan. 23, 1996, Construction and use of synthetic constructs encoding syndecan; Scott Saunders, et al., 530/395; 435/69.1, 69.7, 252.3, 320.1; 536/23.4, 23.5; 935/10, 47, 50, 70 [IMAGE AVAILABLE]

30. 5,482,851, Jan. 9, 1996, Nucleic acid encoding TGF-.beta. and its uses; Rik M. A. Derynck, et al., 435/358, 69.4; 530/350 [IMAGE AVAILABLE]

31. 5,464,815, Nov. 7, 1995, Inhibition of heparin-binding; Steven Chamow, et al., 514/8; 424/85.2; 436/86, 87; 514/21; 530/412 [IMAGE AVAILABLE]

32. 5,457,035, Oct. 10, 1995, Cytokine which is a ligand for OX40; Peter R. Baum, et al., 435/69.5, 252.3, 320.1, 364; 530/351; 536/23.5; 935/9 [IMAGE AVAILABLE]

33. 5,455,165, Oct. 3, 1995, Expression vector encoding hybrid immunoglobulins; Daniel J. Capon, et al., 435/69.7, 252.3, 320.1; 536/23.4 [IMAGE AVAILABLE]

34. 5,449,755, Sep. 12, 1995, Human cyclin E; James M. Roberts, et al., 530/350, 387.1; 536/23.5 [IMAGE AVAILABLE]

35. 5,447,851, Sep. 5, 1995, DNA encoding a chimeric polypeptide comprising the extracellular domain of TNF receptor fused to IgG, vectors, and host cells; Bruce A. Beutler, et al., 435/69.7, 69.5, 320.1, 328, 365; 530/300, 351; 536/23.4 [IMAGE AVAILABLE]

36. 5,444,151, Aug. 22, 1995, **Platelet** **derived** **growth** **factor** **antagonists**; Flemming S. Vassbotn, et al., 530/324, 325, 326, 327, 350, 399, 402, 408; 930/120 [IMAGE AVAILABLE]

37. 5,428,130, Jun. 27, 1995, Hybrid immunoglobulins; Daniel J. Capon, et al., 530/350; 435/69.7; 530/387.1; 536/23.4 [IMAGE AVAILABLE]

38. 5,426,181, Jun. 20, 1995, DNA encoding cytokine-induced protein, TSG-14; Tae H. Lee, et al., 536/23.5; 435/69.1, 252.3, 320.1; 536/23.1 [IMAGE AVAILABLE]

39. 5,418,135, May 23, 1995, Method of inhibiting binding of **PDGF** to a **PDGF** receptor by biosynthetic **PDGF** **antagonists**; Roy H. L. Pang, 435/7.1; 424/143.1; 435/7.2, 7.21, 7.32; 530/324, 350, 399 [IMAGE AVAILABLE]

40. 5,408,040, Apr. 18, 1995, Connective tissue growth factor(CTGF); Gary R. Grotendorst, et al., 530/399; 435/69.4; 530/387.9; 930/120 [IMAGE AVAILABLE]

41. 5,386,013, Jan. 31, 1995, Tumor necrosis factor-induced protein TSG-6; Tae H. Lee, et al., 530/350; 435/69.1; 530/351 [IMAGE AVAILABLE]

42. 5,349,053, Sep. 20, 1994, Chimeric ligand/immunoglobulin molecules and their uses; Nicholas F. Landolfi, 530/351; 424/85.2, 134.1; 530/387.1, 387.3, 387.9, 388.1, 388.25, 389.1, 389.3, 391.1, 391.7, 391.9; 930/141 [IMAGE AVAILABLE]

43. 5,328,837, Jul. 12, 1994, Hepatocyte growth factor protease domain variants; Paul J. Godowski, et al., 435/69.4; 530/399; 536/23.51 [IMAGE AVAILABLE]

44. 5,326,695, Jul. 5, 1994, **Platelet** **derived** **growth** **factor** agonists; Maria Andersson, et al., 435/70.1, 243, 244, 320.1, 365; 530/350, 399; 536/23.5, 23.51 [IMAGE AVAILABLE]

45. 5,316,921, May 31, 1994, Single-chain hepatocyte growth factor variants; Paul J. Godowski, et al., 435/69.4; 530/399; 536/23.51 [IMAGE AVAILABLE]

46. 5,302,519, Apr. 12, 1994, Method of producing a Mad polypeptide; Elizabeth M. Blackwood, et al., 435/69.1, 6, 69.3, 70.21; 530/350, 351; 536/23.1, 23.5 [IMAGE AVAILABLE]

47. 5,284,763, Feb. 8, 1994, Nucleic acid encoding TGF-.beta. and its uses; Rik M. A. Derynk, et al., 435/360, 69.4, 252.3, 320.1; 536/23.5, 23.51 [IMAGE AVAILABLE]

48. 5,234,908, Aug. 10, 1993, Method of treating gastrointestinal ulcers with **platelet** **derived** **growth** **factor**; Sandor Szabo, et al., 514/12, 8, 21 [IMAGE AVAILABLE]

49. 5,225,538, Jul. 6, 1993, Lymphocyte homing receptor/immunoglobulin fusion proteins; Daniel J. Capon, et al., 530/387.3; 424/134.1; 435/69.7; 530/388.73 [IMAGE AVAILABLE]

50. 5,202,428, Apr. 13, 1993, DNA encoding neurotropic growth factor; David Schubert, 435/320.1, 69.51, 325; 530/399; 536/23.1, 23.5 [IMAGE AVAILABLE]

51. 5,168,051, Dec. 1, 1992, Nucleic acid encoding TGF-.beta. its uses; Rik M. A. Derynck, et al., 435/69.4, 69.1, 172.3, 320.1; 935/11 [IMAGE AVAILABLE]

52. 5,155,027, Oct. 13, 1992, Method of producing secreted receptor analogs and biologically active peptide dimers; Andrzej Z. Sledziewski, et al., 435/69.7, 172.3; 530/350, 388.22, 389.3 [IMAGE AVAILABLE]

53. 5,116,964, May 26, 1992, Hybrid immunoglobulins; Daniel J. Capon, et al., 536/23.5; 424/134.1; 435/69.7, 252.3, 320.1; 530/350, 387.3; 536/23.51, 23.53 [IMAGE AVAILABLE]

54. 4,962,091, Oct. 9, 1990, Controlled release of macromolecular polypeptides; Deborah A. Eppstein, et al., 424/85.2, 85.1, 85.4, 85.6, 130.1, 178.1, 184.1, 193.1, 499; 514/2, 21, 964 [IMAGE AVAILABLE]

55. 4,886,747, Dec. 12, 1989, Nucleic acid encoding TGF-.beta. and its uses; Rik M. A. Derynck, et al., 435/69.4, 69.9, 172.1, 172.3, 320.1, 360; 536/23.4, 23.51; 935/11, 34, 70 [IMAGE AVAILABLE]

=> d 36 abs
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US PAT NO: 5,444,151 [IMAGE AVAILABLE] L6: 36 of 55

ABSTRACT:
The invention describes **antagonists** for **PDGF**. The **antagonists** contain amino acids, and may be monomers or dimers. Especially preferred are dimers which bind the **PDGF** receptors, but prevent **dimerization** of the bound receptors. **Dimerization** is necessary for **PDGF** effect, hence the **antagonistic** effect. Also described are nucleic acid sequences for making the **antagonists**, as well as cell lines transfected with the material.

=> d 36 clms

US PAT NO: 5,444,151 [IMAGE AVAILABLE] L6: 36 of 55

CLAIMS:

CLMS(1)

We claim:

1. Isolated peptide **antagonist** for **platelet** **derived** **growth** **factor**, consisting of amino acid sequence:

Ala Asn Phe Leu Val Xaa Xaa Glu Ile Val Arg Lys Lys Pro (SEQ ID NO: 2) wherein the first Xaa is modified tryptophan, and the second Xaa is anywhere from 0 to 35 amino acids.

CLMS(2)

2. The isolated peptide **antagonist** of claim 1, wherein the second Xaa is 0 amino acids.

CLMS(3)

3. The isolated peptide **antagonist** of claim 2, wherein the first Xaa is thioanisolated tryptophan, or a 2-nitrophenyl sulfenyl chloride derivative of tryptophan.

CLMS(4)

4. The isolated peptide **antagonist** of claim 1, wherein the second Xaa is Pro Pro Cys Val Glu Val Gln Leu Arg Pro Val Gln Val Arg Lys Ile, which corresponds to amino acids 7-22 of SEQ ID NO: 5.

CLMS(5)

5. The isolated peptide **antagonist** of claim 4, wherein the first Xaa is thioanisolated tryptophan or a 2-nitrophenylsulfenyl tryptophan derivative.

=> d 39 clms

US PAT NO: 5,418,135 [IMAGE AVAILABLE] L6: 39 of 55

CLAIMS:

CLMS(1)

What is claimed is:

1. A method of inhibiting binding of **platelet**-**derived** **growth** **factor** (**PDGF**) to a **PDGF** receptor on a cell surface, said method comprising the steps of:
a) providing a biosynthetic polypeptide, incapable of **PDGF** activity, said polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO 2 and SEQ ID NO 4 wherein said polypeptide binds to the **PDGF** receptor; and
b) contacting said cell with said polypeptide such that said polypeptide binds said receptor on said cell,
wherein binding of said polypeptide to said receptor inhibits the binding of **PDGF**.

CLMS(2)

2. The method of claim 1, wherein said polypeptide comprises the amino acid sequence set forth in the Sequence Listing as SEQ ID NO:2.

CLMS(3)

3. The method of claim 1, wherein said polypeptide comprises the amino acid sequence set forth in the Sequence Listing as SEQ ID NO:4.

CLMS(4)

4. The method of claim 1 wherein said polypeptide comprises residues 12 through 110 of the amino acid sequences selcted from the group consisting of SEQ ID NO 2 and SEQ ID NO 4.

CLMS(5)

5. The method of claim 1 wherein said polypeptide comprises amino acid residues 12-41 of the Sequence Listing selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:4.

CLMS(6)

6. The method of claim 1 wherein said polypeptide comprises amino acid residues 80-110 of the Sequence Listing selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:4.

CLMS(7)

7. The method of claim 1 wherein said polypeptide is the product of expression of recombinant DNA in a prokaryotic host cell.

CLMS(8)

8. The method of claim 1 wherein said polypeptide is free of glycosylation.

CLMS(9)

9. The method of claim 1 wherein said polypeptide has an amino acid sequence comprising plural blocked **cysteine** residues.

=> d 44 clms

US PAT NO: 5,326,695 [IMAGE AVAILABLE] L6: 44 of 55

CLAIMS:

CLMS(1)

We claim:

1. Isolated **platelet** **derived** **growth** **factor** agonist which binds to **PDGF**-.beta. receptor and comprises amino acids 97-180 of **PDGF**-B **monomer** with the proviso that residues 124 and 133 are not
cysteine

CLMS(2)

2. The agonist of claim 1, wherein at least one of residues 124 and 133 is serine.

CLMS(3)

3. The agonist of claim 1, wherein both of residues 124 and 133 are serine.

CLMS(4)

4. Isolated nucleic acid molecule coding for the agonist of claim 1.

CLMS(5)

5. Plasmid containing the isolated nucleic acid molecule of claim 4.

CLMS(6)

6. Cell line transfected with the nucleic acid molecule of claim 4.

CLMS(7)

7. Cell line transfected with the plasmid of claim 5.

CLMS(8)

8. Method for causing receptor **dimerization** and autophosphorylation in a cell having **PDGF**-.beta. receptors on its surface comprising administering to a cultured cell having **PDGF**-.beta. receptor on its surface an amount of the agonist of claim sufficient to cause receptor **dimerization** and autophosphorylation in said cell.

=> d 9 clms

US PAT NO: 5,607,918 [IMAGE AVAILABLE] L6: 9 of 55

CLAIMS:

CLMS(1)

What is claimed is:

1. An isolated protein having the property of promoting proliferation of endothelial cells or mesodermal cells, said isolated protein comprising a sequence of amino acids selected from the group consisting of the amino acid sequence of FIG. 1 (SEQ ID NO:2), the amino acid sequence of FIG. 2 (SEQ ID NO:3), the amino acid sequence of FIG. 4 (SEQ ID NO:5), the amino acid sequence of FIG. 6 (SEQ ID NO:7), the amino acid sequence of FIG. 8 (SEQ ID NO:9), and the amino acid sequence of FIG. 11 (SEQ ID NO:11).

CLMS(2)

2. An isolated protein according to claim 1, wherein said protein comprises the amino acid sequence of FIG. 1 (SEQ ID NO:2).

CLMS(3)

3. An isolated protein according to claim 1, wherein said isolated protein comprises the amino acid sequence of FIG. 2 (SEQ ID NO:3).

CLMS(4)

4. An isolated protein according to claim 1, wherein said isolated protein comprises the amino acid sequence of FIG. 4 (SEQ ID NO:5).

CLMS(5)

5. An isolated protein according to claim 1, wherein said isolated protein comprises the amino acid sequence of FIG. 6 (SEQ ID NO:7).

CLMS(6)

6. An isolated protein according to claim 1, wherein said isolated protein comprises the amino acid sequence of FIG. 8 (SEQ ID NO:9).

CLMS(7)

7. An isolated protein according to claim 1, wherein said isolated protein comprises the amino acid sequence of FIG. 11 (SEQ ID NO:11).

CLMS(8)

8. An isolated protein according to claim 1, wherein said isolated protein is a mammalian protein.

CLMS(9)

9. An isolated protein according to claim 8, wherein said mammalian protein is a murine protein.

CLMS(10)

10. An isolated protein according to claim 8, wherein said mammalian protein is a human protein.

CLMS(11)

11. An isolated protein according to claim 1, wherein said isolated protein promotes proliferation of vascular endothelial cells.

CLMS(12)

12. An isolated protein produced by expression of a DNA selected from the group consisting of the DNA of FIGS. 1 and 2 (SEQ ID NO:1), the DNA of FIG. 3 (SEQ ID NO:4), the DNA of FIG. 5 (SEQ ID NO:6), the DNA of FIG.
7 (SEQ ID NO:8), the DNA of FIG. 10 (SEQ ID NO:10), and DNA which hybridizes under stringent conditions with at least one of the foregoing DNA sequences.

CLMS(13)

13. A pharmaceutical composition comprising an effective endothelial or mesodermal cell proliferation promoting amount of an isolated protein according to claim 1, and at least one pharmaceutical carrier or diluent.

=> d 39 kwic

US PAT NO: 5,418,135 [IMAGE AVAILABLE] L6: 39 of 55
TITLE: Method of inhibiting binding of **PDGF** to a **PDGF** receptor by biosynthetic **PDGF** **antagonists**

ABSTRACT:
Disclosed are polypeptides which **antagonize** the activity of **platelet**-**derived** **growth** **factor** (**PDGF**). These polypeptides include an amino acid sequence sufficiently duplicative of at least a portion of the amino acid sequence of an A chain of **PDGF** such that the polypeptides bind a cell membrane-bound receptor for native **PDGF** on a cell that responds biologically to the binding of **PDGF**. The binding of the **antagonist** to the receptor is effective to inhibit **PDGF** binding and activity. Also disclosed are methods of preparing and using these **antagonists**.

SUMMARY:

BSUM(4)

A high concentration of **platelet**-**derived** **growth** **factor** (**PDGF**) is found at the site of the lesion, and later, in the fibrous plaque (Barrett et al. (1988) Proc. Natl.. .

SUMMARY:

BSUM(5)

Native **PDGF** is a dimeric molecule consisting of two polypeptide chains, one or more of which appear to be glycosylated. The two. .

SUMMARY:

BSUM(6)

Biologically active **PDGF** can exist as an AA or BB homodimer, having a molecular weight of about 35,000 daltons (35 kD) or about 32 kD, respectively, or can take the form of an AB **heterodimer** having a molecular weight of about 34 kD. The human **PDGF** **dimer** is glycosylated and processed post-translationally into a three-dimensional conformation that is biologically active. This conformation is maintained by relatively weak noncovalent hydrogen bonds, hydrophobic and charge interactions, and strong covalent bonds between sulfur atoms in **cysteine** residues. The **PDGF** **dimer** has eight such disulfide linkages which exist both between chains (interchain bonds) and within the same chain (intrachain bonds). Reduction of either the AA or BB **dimer** into its component monomeric chains destroys all biological activity.

SUMMARY:

BSUM(7)

Different cell types are known to elicit different dimeric forms of **PDGF**. In fact, many of the cells intimately involved in the formation of the plaque produce and secrete various forms of **PDGF**. For example, platelets aggregating at the site of initial injury at the endothelial lining release **PDGF** AB. Macrophages produce **PDGF** BB, and SMC and
endothelial cells produce **PDGF** AA.

SUMMARY:

BSUM(8)

Platelet-**derived** **growth** **factor** has been postulated to be the etiological agent in atherosclerosis (see e.g., Rutherford et al. (1976) J. Cell. Biol. 69:196-203; Friedman et al. (1977) J. Clin. Invest. 60:1191-1201). The released **PDGF** is able to chemotactically recruit fibroblasts, monocytes, glia, and smooth muscle cells to migrate to the site of the wound. The released **PDGF** also acts as a mitogen by stimulating DNA synthesis in these cells, thereby increasing their proliferation rate. Quiescent SMC normally found in nonembryonic arterial walls, becomes synthetic and proliferative upon stimulation with the **PDGF** produced by endothelial cells, macrophages, and platelets. In this active state, SMC, themselves, produce **PDGF** AA which in turn, activates quiescent SMC.

SUMMARY:

BSUM(9)

It has been hypothesized that inhibiting the activity of **PDGF** may inhibit or reverse the formation of atherosclerotic plaques. To that end, a number of different molecules were tested as inhibitors or **antagonists** of **PDGF**. For example, fenofibrate (Kloer (1987) Am. J. Med. 83(B):3-8) and retinoic acid (Mordan (1989) Cancer Res. 49:906-909) inhibit **PDGF**-dependent stimulation of DNA synthesis. Monoclonal antibody C3.1 (Kawahara et al. (1987) Biochem. Biophys. Res. Commun. 147:839-845) and 5-methyl-7-diethylamino-s-triazolo (I,5-a) pyrimidine (Ohnishi et al. (1983) Life Sci. 31:2595-2602; Tiell et al. (1983) Artery 12:33-50) are **PDGF** **antagonists**. Interferon inhibits **PDGF**-induced protein synthesis in fibroblasts (Zagari et al. (1988) Biochem. Biophys. Res. Commun. 150:1207-1212) and inhibits the mitogenic effect of **PDGF** on fibroblasts (Hosang (1988) J. Cell. Physiol. 194:396-404). Suramin binds to **PDGF** and inhibits its biological activity (Hosang (1985) J. Cell. Biochem. 29:265-273), and protamine inhibits the binding of **PDGF** to its receptor (Huang (1984) J. Cell. Biol. 26:205-220).

SUMMARY:

BSUM(10)

The object of this invention is to inhibit the binding of **PDGF** to its receptors on responsive cells, and thus to inhibit the subsequent biological activities triggered by the binding of active **PDGF** to its receptors. It is also an object of the present invention to inhibit the formation of atherosclerotic lesions and fibrous plaques by inhibiting the biological activity of **PDGF**. Another object is to stop and/or to reverse the progression of atherosclerosis. Another object is to inhibit the proliferation of. . .

SUMMARY:

BSUM(12)

This invention provides methods of **antagonizing** the activity of **platelet** **derived** **growth** **factor** (**PDGF**) with the use of
polypeptides or **antagonists** having no **PDGF**-related biological activity, but having the ability to compete with biologically active forms of **PDGF** for **PDGF** receptors on cells. The polypeptides have an amino acid sequence sufficiently duplicative of at least a portion of an A chain of a biologically active form of **PDGF** such that it binds a cell membrane-bound receptor for native **PDGF** on a cell that responds biologically to the binding of **PDGF**. The binding of the polypeptide of the invention to a **PDGF** receptor effectively inhibits the binding of **PDGF** thereto, and in this way blocks the initiation of the biological activities triggered by **PDGF** binding. In some aspects of the invention, the polypeptide has at least 70% homology with residues 12-110 of the amino acid sequences for A chains of **PDGF** set forth in the sequence listing as SEQ ID NOS:1 and 3.

SUMMARY:

BSUM(13)

The polypeptide **antagonists** provided by this invention may be free of glycosylation and remain in monomeric form as they may be designed to lack the sulfhydryl group cross-linking sites prerequisite to form a biologically active **PDGF** **dimer**. In accordance with this aspect of the invention, the polypeptide may take the form of a **cysteine**-free or **cysteine**-blocked, full length or truncated A chain of **PDGF** such as an endothelial form of the A chain (see, e.g., SEQ ID NO:1) or a glioma form of the. . . A chain (see, e.g., SEQ ID NO:3). Alternatively, the polypeptide may comprise a mutein, analog, or truncated analog of a **PDGF** A chain. **Cysteine** residues of the polypeptide may be blocked, for example, by conventional methods including sulfonation, pyridylethylation, or carboxymethylation.

SUMMARY:

BSUM(14)

Peptide . . . of a native A chain or analog or mutein thereof retaining have at least some residual specific affinity for a **PDGF**-specific receptor also are useful as **PDGF** **antagonists**. These fragments may assume a monomeric form because some or all of their Cys residues have been blocked or replaced. . . with amino acids incapable of forming disulfide bonds. Alternatively, these fragments may be disulfide-bonded to a second polypeptide not having **PDGF** biological activity. Preferably, the fragment has an amino acid sequence homologous with a portion of a native endothelial or glioma species of a **PDGF** A chain, and more preferably, includes amino acid residue 80-110 or residues 12-41 thereof (see, e.g., SEQ ID NOS:1 and. . .

SUMMARY:

BSUM(16)

Lastly, the invention provides a method of preparing these **antagonist** polypeptides including the steps of culturing a cell transfected with a DNA sequence encoding the polypeptide and capable of expressing. . .

DRAWING DESC:

DRWD(3)

FIGS. . . . compare diagrammatic representations of various embodiments of the invention (FIGS. 1B-1G) with a highly diagrammatic representation of a disulfide-bonded, native **PDGF** **dimer** (FIG. 1A);

DRAWING DESC:

DRWD(4)

FIG. . . . a schematic representation of a recombinant DNA of the invention comprising a structural gene encoding an endothelial A chain of **PDGF**, the corresponding amino acid sequence, and a restriction map;

DRAWING DESC:

DRWD(5)

FIG. . . . recombinant DNA of the invention including a vector-derived polylinker region and a structural gene encoding a glioma A chain of **PDGF**, the corresponding amino acid sequence, and a restriction map; and

DRAWING DESC:

DRWD(6)

FIG. . . . and the corresponding amino acid sequence for the LE peptide. This operator/promoter - leader DNA is preferred for expressing the **PDGF** **antagonists** of FIGS. 1, 2, and 3 in E. coli.

DRAWING DESC:

DRWD(8)

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=> d 39

39. 5,418,135, May 23, 1995, Method of inhibiting binding of **PDGF** to a **PDGF** receptor by biosynthetic **PDGF** **antagonists**; Roy H. L. Pang, 435/7.1; 424/143.1; 435/7.2, 7.21, 7.32; 530/324, 350, 399 [IMAGE AVAILABLE]

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